# Scanning electron microscopy of three types of ectomycorrhizae formed on *Eucalyptus nova-anglica* in the southeastern United States[1]

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Three different types of ectomycorrhizae found in the Southeastern United States on *Eucalyptus nova-anglica* and identified as being formed by *Cenococcum geophilum*, *Pisolithus tinctorius*, and *Scleroderma geaster* were examined with a scanning electron microscope (SEM). In overall appearance the three types of mycorrhizae could be distinguished easily from each other, particularly *C. geophilum*, which had a relatively smooth, undulate surface in comparison with the other two types. Detailed descriptions and comparisons of these ectomycorrhizae are given and the value of SEM in characterizing ectomycorrhizae is discussed.

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On a examiné au microscope électronique à balayage (MEB) trois types d'ectomycorhizes formés respectivement par *Cenococcum geophilum*, *Pisolithus tinctorius* et *Scleroderma geaster* sur les racines d'*Eucalyptus nova-anglica* dans le sud-est des États-Unis. Les trois types de mycorhizes se distinguent facilement par leur allure générale, surtout celle formée par *C. geophilum* qui a une surface relativement unie et ondulée par comparaison aux deux autres types. Ces ectomycorhizes sont décrites en détail et comparées et on discute l'utilité du MEB pour caractériser les ectomycorhizes.

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## Introduction

Numerous types of ectomycorrhizae have been described in detail for species of *Eucalyptus* (Chilvers and Pryor 1965; Chilvers 1968). Chilvers (1968) used four terms to describe the hyphal structure of the mantle as seen with light microscopy: felt prosenchyma, net prosenchyma, irregular synenchyma, and regular synenchyma. These terms characterize both the developmental stages and intermantle structure of eucalypt ectomycorrhizae. Seviour *et al.* (1978) published scanning electron micrographs (SEM) of the surface features of eucalypt mycorrhizae but "no micrographs were obtained showing tissue organization of regular synenchyma type."

The purpose of this study was to elucidate by SEM different morphological and anatomical detail of three different types of eucalypt ectomycorrhizae found in the southeastern United States. We present electron micrographs of these types and describe their morphology, details of external and internal mantles, and sectional profiles of the mantle–root interface.

## Materials and methods

One basidiocarp each of *Pisolithus tinctorius* (Pers.) Coker & Couch and *Scleroderma geaster* Fr. was collected along with the adhering roots of *Eucalyptus nova-anglica* Deane & Maiden from a eucalypt planting along the entrance road to the Southlands Experiment Forest (International Paper Co., Bainbridge, GA). The collection was made in August of 1977 and represents part of a larger study of eucalypt species in the Southeastern United States (Rose 1979). Roots and soil were separated from the base of each basidiocarp and placed in jars containing weak FAA (450 mL of 95% ethanol, 450 mL of distilled water, 50 mL of glacial acetic acid, 50 mL of 40% formalin). The *Cenococcum geophilum* Fr. ectomycorrhizae were found among the *P. tinctorius* collections.

Sectional views were obtained either by hand sectioning the ectomycorrhizae with a razor blade, or sectioning them on a freezing microtome. The sectioned materials as well as intact ectomycorrhizae preserved in FAA were subsequently dehydrated in ethanol and a graded ethanol–Freon series, and critical-point dried in Freon 13. The dried specimens were mounted on stubs, coated with gold, and observed and photographed with an ETEC Autoscan electron microscope.

## Results and discussion

Chilvers (1968), Trappe (1971), and Seviour *et al.* (1978) have provided detailed descriptions of *C. geophilum* mycorrhizae. In this study the SEM's show in more detail many of the features previously described, especially the irregular synenchyma of the *C. geophilum* mantle.

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The other two ectomycorrhizal types had generally similar tissues to those described for eucalypts in Australia (Chilvers 1968) but were noticeably different in certain details. For example, neither *P. tinctorius* nor *S. geaster* ectomycorrhizae showed any hyphal ornamentations similar to those reported by Seviour *et al.* (1978). Also, the mantles of both types appeared to have more loosely woven prosenchymatous tissues than those shown in the SEM study of Seviour *et al.* (1978).

The greater clarity and three dimensional detail of our SEM make it difficult to apply terminology developed by Chilvers (1968) for light microscope descriptions of eucalypt ectomycorrhizae. Similarly, other commonly used descriptive terms such as loose (how loose?), cottony, woolly, and weft were difficult to use in comprehensively describing prosenchymatous tissue.

The following surface descriptions combine both light microscopy and low power SEM. All other descriptions come directly from the SEM micrographs; observations of mantle structure were made at an angle perpendicular to the mycorrhiza surface. In this way prosenchyma and synenchyma tissue types were distinguished on the basis of hyphal length, branching, and degree of compaction.

Detailed descriptions of these three ectomycorrhizal types are as follows.

Cenococcum geophilum *plus* Eucalyptus nova-anglica

*Surface features*

Under light microscopy these ectomycorrhizae were black with numerous bristlelike emanating hyphae. They formed both monopodial and ramiform types, usually with only one branch. The surface texture was smooth-undulate. SEM (Fig. 1) showed the undulate nature to be caused by closely packed hyphae (2.5–3.4 μm in diameter), often aligned in parallel rows radiating from stellate configurations. However, mantles of *C. geophilum* ectomycorrhizae seemed to fit the description "featureless appearance" of Seviour *et al.* (1978) because the mantles were relatively smooth without distinct hyphal contour. Figure 2 shows the early stages of mantle development. A few parallel hyphae perpendicular to adjoining hyphae appear to form the center of a stellate configuration. Although the characteristic emanating hyphae were broken off in the preparation shown in Fig. 1, they remained in other preparations.

*SEM of sectional views*

In sectional views, the inner mantle hyphae appeared as synenchyma tissue in places (Fig. 3). Hyphal walls were closely packed and appeared fused, lacking intercellular spaces. A section through the parallel-oriented mantle hyphae showed these fungal cells to be isodiametrically arranged. In longitudinal sections the mantle was usually 6–10 hyphal cell layers thick (Fig. 4).

Hartig net was clearly observed in some of the sectional views. The mantle was occasionally separated from the root (this may have been a fixation artifact, as the tissues were very delicate when dried).

Pisolithus tinctorius *plus* Eucalyptus nova-anglica

*Surface features*

These ectomycorrhizae were brown to golden-brown with some emanating hyaline hyphae. The form was both monopodial and ramiform with irregular or regular open pinnate development. Most ramiform structures appeared tortuous. Mantle texture was smooth to cottony. SEM showed the outer mantle hyphae to be tightly interwoven and closely adhering to the root surface and to each other (Fig. 5).

*SEM surface views of outer mantle hyphae*

The outer hyphae were loosely appressed and interwoven on the surface of the mycorrhiza (Fig. 6). Open-looping clamp connections occurred frequently, but anastomoses among the hyphae (mean diameter 3.2 μm) were infrequent.

*SEM surface views of inner mantle hyphae*

Prosenchyma hyphae formed a tight weft with frequent anastomoses in the inner mantle (Fig. 7). Hyphae had an appressed appearance. Open-looping clamp connections were frequent and closely spaced.

*SEM cross sections*

The outer mantle felt prosenchyma graded to an irregular synenchymatous inner mantle, as seen in Fig. 8. Hartig net hyphae tightly conformed to the outer cortical cells of the root.

Scleroderma geaster *plus* Eucalyptus nova-anglica

*Surface features*

These ectomycorrhizae were white with a dense thick woolly mantle. The form was either monopodial or ramiform, with irregular to regular pinnate development. Most ramiform structures appeared tortuous. With SEM, the outer mantle (Fig. 9) appeared as loosely connected fine threads producing a woolly surface texture.

*SEM surface views of outer mantle hyphae*

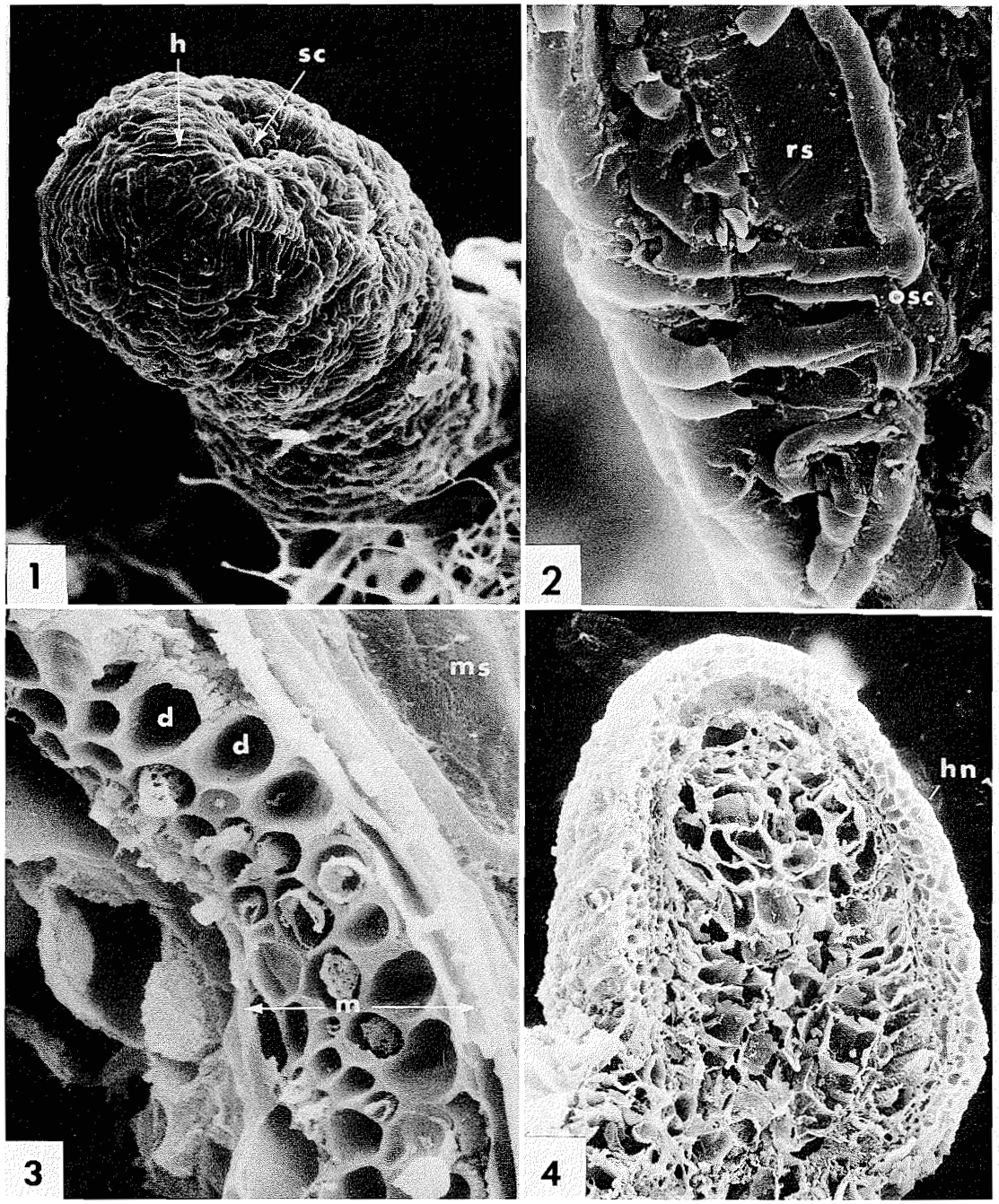
Interwoven felt prosenchyma hyphae formed the outer mantle. Anastomoses among hyphae were common. Hyphal diameter ranged from 1.4 to 2.4 μm and open-looping clamp connections were infrequent (Fig. 10).

*SEM surface views of inner mantle hyphae*

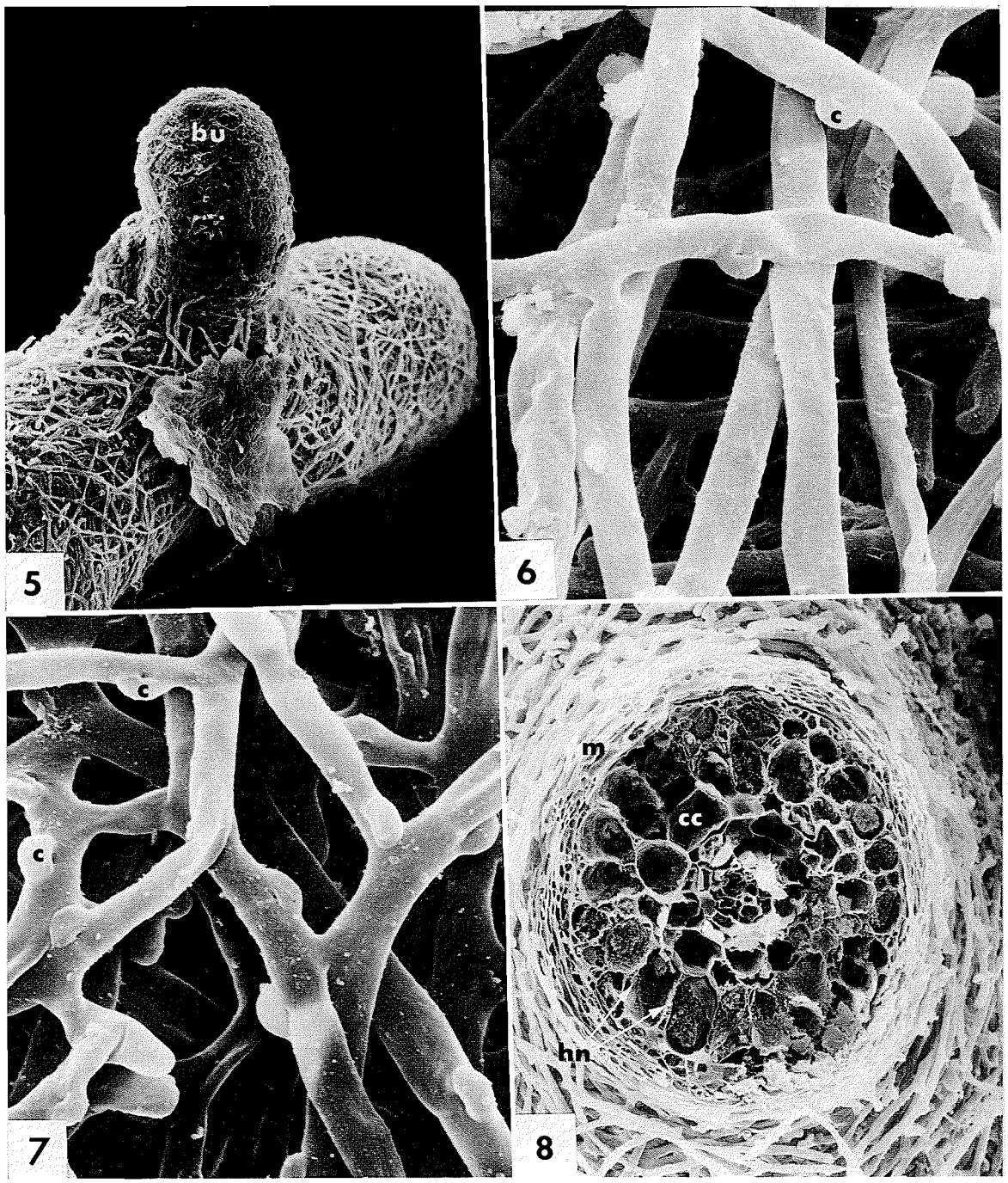
Irregular synenchyma formed a tight weft in the inner mantle (Fig. 11). Anastomoses occurred frequently, and the hyphae had an appressed appearance.

*SEM of cross sections*

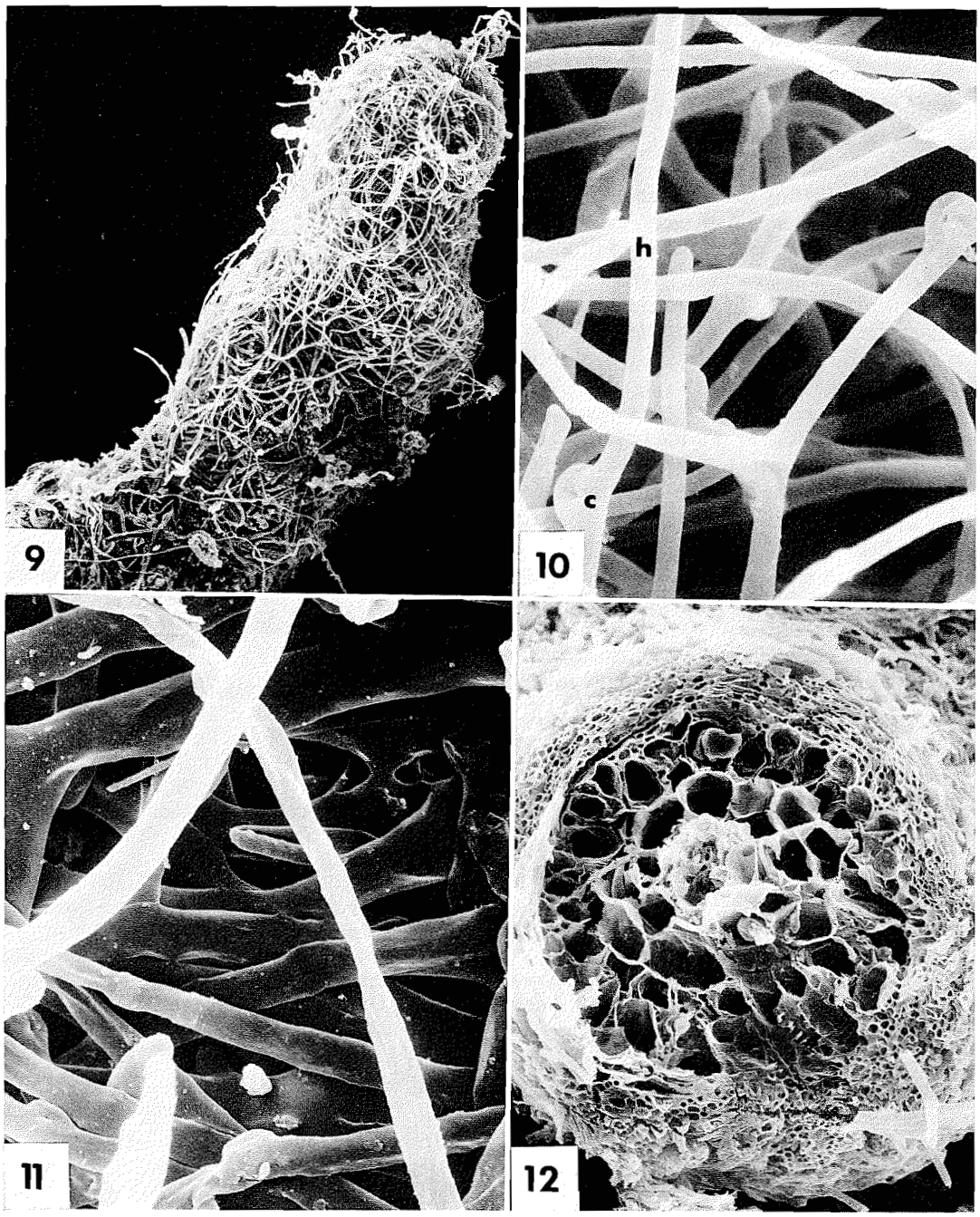
The felt prosenchyma hyphae of the outer mantle




FIGS. 1–4. SEM micrographs of *Cenococcum geophilum* in ectomycorrhizal relationship with roots of *Eucalyptus nova-anglica*. Fig. 1. View of the ectomycorrhiza showing the smooth-undulating surface texture, center of stellate configuration (*sc*) with radiating parallel hyphae (*h*). ×300. Fig. 2. View of early development of parallel hyphae on the root surface (*rs*) may represent the origin of a stellate configuration (*osc*). ×2100. Fig. 3. Sectioned mycorrhiza showing the isodiametry of parallel hyphal cells (*d*) in the mantle (*m*); *ms*, mantle surface. ×2300. Fig. 4. Longitudinal section through the mycorrhiza showing the mantle–root interrelationship. Some Hartig net hyphae (*hn*) are visible. ×400.




FIGS. 5–8. SEM micrographs of *Pisolithus tinctorius* in the relationship with *Eucalyptus nova-anglica*. Fig. 5. View of the ectomycorrhiza showing the smooth, appressed weftlike surface features; the branch upward (*bu*) has an irregular synenchyma surface. ×200. Fig. 6. Close-up view of the outer mantle hyphae with a loose, weft arrangement and open-looping clamp connections (*c*). ×2600. Fig. 7. View of net prosenchyma inner mantle, with clamp connections (*c*) and anastomosing hyphae (*a*). ×2600. Fig. 8. Cross-section profile of a mycorrhiza showing the mantle, Hartig net, and root cortical cells (*cc*). ×500.




FIGS. 9–12. SEM micrographs of *Scleroderma geaster* in relationship with *Eucalpytus nova-anglica*. Fig. 9. View of this ectomycorrhiza showing the woolly mantle texture. ×200. Fig. 10. Close-up view of the thick outer mantle hyphae and clamp connections. ×2600. Fig. 11. View of the tight-weft inner mantle. ×2600. Fig. 12. Cross-section profile of a mycorrhiza showing the mantle–root interrelationship. ×500.

intergraded to irregular synenchyma in the cross sectional views of *S. geaster* (Fig. 12). Hartig net hyphae penetrated usually one tier of cortical cells.

*Comparative descriptions of ectomycorrhizal types*

*Overall appearance*

These ectomycorrhizal types were easily distinguished from one another by their overall appearance. Surface hyphae of *C. geophilum* were most nearly unique in character and thus easily discerned (Fig. 1).

*SEM surface views of outer mantle hyphae*

The outer mantle hyphae of *P. tinctorius* and *S. geaster* differed in relative size and branching habit. *Pisolithus tinctorius* (Fig. 6) displayed a weftlike pattern not manifested by *S. geaster* (Fig. 10). Anastomoses were more common among the outer mantle hyphae of *S. geaster* than of *P. tinctorius*. Clamp connections did not differ markedly between these two fungi, although *P. tinctorius* tended to have more than *S. geaster*.

*Inner mantle hyphae*

The hyphae of *P. tinctorius* (Fig. 7) had a tightly woven net prosenchyma, which was not evident with *S. geaster* (Fig. 11). Mantle hyphae of *S. geaster* always appeared to grade from felt prosenchyma tissue directly to irregular synenchyma tissue when seen at an angle perpendicular to the surface of the mycorrhiza.

*Cross sections*

The irregular synenchymatous mantles and the Hartig net hyphae of *P. tinctorius* and *S. geaster* ectomycorrhizae were too similar to permit differentiation. The mantles formed by *C. geophilum* were always composed of thick-walled hyphae forming irregular synenchyma tissue.

## Conclusion

The characterization of ectomycorrhizae by use of SEM is promising, but should be used with some caution. Our results show that two distinct ectomycorrhizae can have strikingly similar hyphal and cross-sectional profiles. Even an easily recognized mycorrhiza such as that formed with *C. geophilum* can have different developmental stages, which can complicate identification.

Light microscopy and SEM are complementary methods for characterizing mycorrhizae. It is relatively simple to fix a sample in FAA, observe and photograph with a light microscope, and subsequently prepare the same sample for SEM. Characterization can thus proceed along a series of steps from assessment of branching and color with the light microscope to quantitative measures of hyphal diameter and anastomoses with SEM. When both methods are used in consort the characterization of ectomycorrhizae can be more precisely described.

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